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INTERFERENCE OF ANIMAL SOURCE AMMONIA WITH EXPOSURE CHAMBER ATMOSPHERES CONTAINING ACID PARTICULATE FROM AUTOMOBILE EXHAUST

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A study was designed to test the effect of animal source ammonia on some component concentrations in test atmospheres produced to measure the health effects of pollutants in the exhaust emissions of gasoline-powered engines equipped with oxidative catalyst converters. The dominantly acidic nature of the exhaust particulate phase required an evaluation of test chamber concentrations of the alkaline components contributed by the test animals. The animal population in the chamber was critical in maintaining the essential integrity of the pollutant atmosphere. Measures to minimize the production of the animal-produced interferent(s) were helpful in attenuating the interfering effect.

INTRODUCTION

In the study of toxic effects of atmospheric pollutants inhaled by animals in test exposure chambers, the maintenance of the integrity of the toxic component is necessary. The experimental animals contribute components to the atmosphere which may have considerable impact upon the overall composition of the exposure atmosphere. Fraser *et al.* (1959) sounded the warning about the effects of components common to chamber atmospheres. Knott and Malanchuk (1969) established the presence of a substantial amount of an animal-contributed reaction product in a study planned for the effects of a specific gas-particulate system. A most serious case of such change occurs when a considerable proportion of the key component of the study atmosphere is converted into a product having completely different physical, chemical and toxicological characteristics.

When plans were being made for an inhalation study related to health effects of automobile exhaust emissions, it was recognized that the acidic nature of the aerosol component could be seriously attenuated by the ammonia and other alkaline compounds produced from the excreta of the test

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animals. The aerosol was sulfuric acid of 40-50 percent aqueous droplets, a phase condition in the air-diluted exhaust emissions from an engine equipped with an oxidative catalyst in the exhaust system. Of some concern, also, was the ammonia in the exhaled air of the animals in a chamber with low air flow.

Therefore, a preliminary study was conducted to evaluate the impact of the interaction of alkaline components of the metabolic products of various numbers of animals (rats) with the acidic atmosphere in the exposure chambers.

The study consisted of three phases, each phase one week long. The exposure chambers were monitored for certain dominant components of the ammonia-sulfuric acid reaction system.

In Phase I, the effect of the number of animals in an exposure chamber upon the ammonia gas concentration in that chamber atmosphere was examined. In Phase II, the impact of the ammonia concentrations produced by different animal populations upon the acid content of the auto exhaust components introduced into the exposure chambers was measured. In Phase III, steps to reduce ammonia production were evaluated; the investigative effort was concentrated on the results of using chemically-treated paper[1] placed beneath the animal cages. The treated paper not only trapped animal droppings for subsequent easy removal during the cleaning operations but also deterred the production of ammonia from *excreta*.

EXPOSURE SYSTEM

The exposure chambers were the Laskin type, with an exposure zone capacity of 1.25m³ (44 cubic feet), as described by Hinners *et al.* (1968). Standard air flow rate was 0.31m³/min (11 cfm) or 15 air changes per hour through each chamber with a negative static pressure of 7.62 mm (0.3 inch) water. A water spray ring at the bottom of the exposure zone was used to flush the bottom cone section of the exposure chamber.

Three shelf levels in each chamber were available for holding the animal cages which were distributed as evenly as possible over the three levels.

Temperature and humidity in the reference, clean-air chambers were controlled at 21.1 to 22.2°C (70 to 72°F) and 50 to 60% relative humidity. Temperatures in the animal-occupied chambers were somewhat elevated, according to the number of animals present, generally by 1.1 to 1.7°C (2-3°F) when "fully" occupied. The humidity was increased as a result of water in combustion products of the auto exhaust emissions and of the greater number of animals in the chambers. Thus, with the maximum load of 36 animals in a chamber the relative humidity was elevated by 10 to 15% over that in the chambers with 6 to 12 animals.

ANALYTICAL PROCEDURES

Atmospheric samples were collected for measurements primarily of ammonia gas and of ammonium, sulfate, and hydrogen ions in the particulate (aerosol) state.

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[1]Deotized animal cageboard, Upjohn Co., Kalamazoo, MI. Reference to trade names does not imply endorsement by the U.S. Government.

Ammonia gas samples were collected at 2-lpm in 10 to 30 ml deionized water, for a total of 20 to 60 liters of the chamber atmosphere. A slightly modified version of the Weatherburn (1967) method was used to analyze for ammonia: 5 ml portions of the phenol-sodium nitroprusside reagent and of the sodium hydroxide - sodium hypochlorite reagent were added to a measured volume of the ammonia solution sample; the sample mix was heated at 37°C for 15 minutes and read at 625 μm on a Beckman ACTA V spectrophotometer against a reagent blank.

Particulate samples from approximately one cubic meter of the exposure chamber atmosphere were collected on teflon-coated and on quartz fiber filters, 47mm D, at a sampling rate of 8-lpm for two hours. Those from the air-dilution tube (immediately after the exhaust pipe) were collected on 142 mm quartz filters for 4 to 8 hours at 14-lpm. The filters were weighed for particulate mass determinations immediately after sampling. The entire 47mm disc, or a quarter section of the 142mm filter, was placed in 60 ml of deionized distilled water for extraction of the trapped particulate material. The aqueous extract was analyzed for total ion concentration by conductance measurements and for hydrogen ion by pH meter readings. Subsequently, the solutions were analyzed for sulfate by the barium chloranilate method as outlined by Kufta (1972), for ammonium by the phenol-hypochlorite reaction as described by Weatherburn (1967), and for nitrate by hydrazine reduction according to a manual adaptation of a method defined by Kamphake *et al.* (1967).

TESTING PROGRAM — PHASE I

A total of 6, 12, or 36 adult rats, with three animals per cage, were placed in each of three different exposure chambers on a Friday afternoon. Chamber airflow was established at the standard 0.31m³/min (11 cfm) rate (a change every four minutes for the 44 cubic foot chamber). Chambers were flushed with water from the spray ring, down the bottom cone sections daily, at approximately 9 A.M.

Initially, two days were used to promote the development of an atmospheric condition dependent only upon the presence of the animals in the chamber, before any samplings of the atmosphere were made. The next day (identified as Day 1 on Fig. 1), and daily for the rest of the week, several gas samples of each chamber atmosphere were collected and analyzed for ammonia. A spot check filter sample from the 36-animal chamber showed negligible particulate present; that situation was expected, and such filter samplings were discontinued for the rest of that phase of the study.

Early on Day 4 the chamber air flow was reduced by a half to test the effect of the sweeping action of the air flow upon the concentration level of diffused ammonia.

The plot of average daily ammonia concentrations (Fig. 1) showed stable low levels in the chambers with the samll, 6-animal population (Chamber S) and with the medium, 12-animal population (Chamber M). However, Chamber L, with the large, 36-animal population, showed a gradual increase in ammonia during the first three days of monitoring, followed by a sharp

increase on the fourth day that reached a concentration of nearly 25 ppm (17.4 mg/m³) on the fifth and final day. The abrupt increase on the last two days occurred at the same time that the air flow through each of the three chambers was reduced to half the normal flow prevailing during the earlier period. The reduced air flow apparently had no significant effect upon the established low ammonia levels in the chambers with 6 and 12 animals, S and M. Apparently there developed a threshold condition involving number of animals and amount of ammonia produced such that a high(er) enough flow rate of clean supply air through the chamber could exert a sufficient degree of flushing action upon the chamber atmosphere to maintain an ammonia level in equilibrium with that flow rate. That was evident in the case of Chamber L with its large load of animals. A return to the standard 0.31m³/min (11 cfm) flow at the end of the study (Day 5) brought the ammonia level in Chamber L with its 36 animals back to about the level existing on day 3 (that result not shown in Fig. 1).

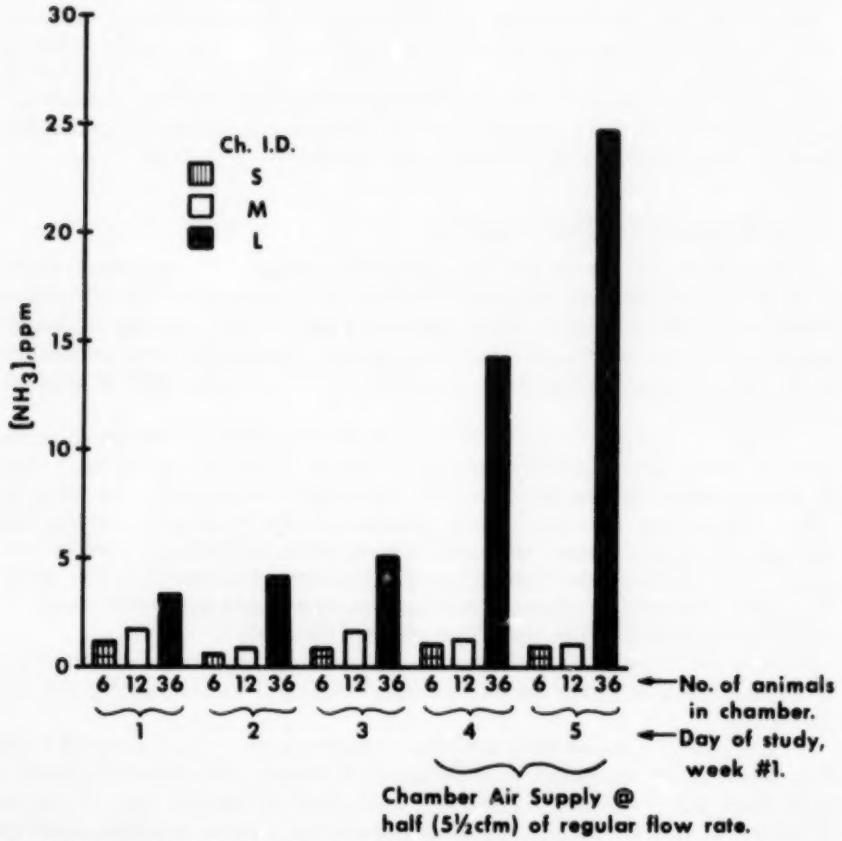

FIGURE1. Ammonia (gas) concentrations in exposure chambers, Phase I.

TESTING PROGRAM — PHASE II

The same arrangement of the 6, 12, and 36 rats in the three exposure chambers was continued for a week of exposure to an atmosphere that consisted of irradiated, dilute (10/1 of air/exhaust) auto exhaust. Chamber flow rate was the same as in Phase I. Exposure to the exhaust emissions atmosphere was maintained through 24 hours per day. In addition to gas samples collected for measurement of ammonia, filter samples were collected and analyzed for acidity, sulfate, ammonium, and nitrate levels.

At midweek, the animals were redistributed among the chambers so that an equal number of 18 were held in each of the three chambers. Because the animal cages at the upper level in Chamber L (large, 36-animal population) had been cleaner than those at the lower levels, the redistribution of animals (cages) from Chamber L to Chamber S and M was arranged so that each of those chambers would have an equal number of the cleaner cages.

A plot of the gaseous ammonia concentrations (Fig. 2) showed that the concentration during the early half of the week was less than 0.5 ppm (0.35 mg/m^3) in those chambers having 6 and 12 animals, and increased to 1 to 2.5 ppm (0.70 to 1.74 mg/m^3) when there were 18 animals in each chamber during the latter half of the week. Ammonia in the 36-animal chamber swelled to 67.2 ppm (46.8 mg/m^3) by mid-week as an extension of the continuously increasing trend of the previous weeks' development, then decreased to 15 ppm (10.4 mg/m^3) and eventually to 8 ppm (5.6 mg/m^3) when the population was reduced to only 18 animals during the latter half of the week.

Figure 3 shows the concentration of the acid component in the filter-collected particulate, based on the sum of the hydrogen (H^+) and the ammonium (NH_4^+) cations measured. Essentially all of the particulate was accounted for in the calculated hydrogen (sulfuric acid) and ammonium sulfates. The acidity of the particulate, based on the total of hydrogen and ammonium ions, averaged about 80 mol percent in the chambers with 6 and 12 rats, S and M, during the first two days and decreased to near zero with the increase of animal number to 18 in each chamber during the remainder of the week. In Chamber L, the acidity remained nearly zero with the thirty-six rats and also with the reduced number of 18.

The consistency of the neutralizing effect of the animal excretion products was seen in the pH values determined on the aqueous extracts of the samples of particulate collected during that week. Except for the samples taken from the 6- and 12- animal chambers in the early part of the week, which yielded extracts of about pH 3, all extracts were essentially neutral solutions, of pH 5.5-6.0.

TESTING PROGRAM - PHASE III

Clean (i.e., washed) rats, 24 and 30 in number, were introduced into the two chambers, S and M, respectively, at the start of Phase III. The exposure chambers had already been thoroughly hosed down with hot water. The flow rate of 0.31 m^3/min (11 cfm) through each chamber was resumed. Chemically-treated paper pads were placed under the animal cages of the upper level

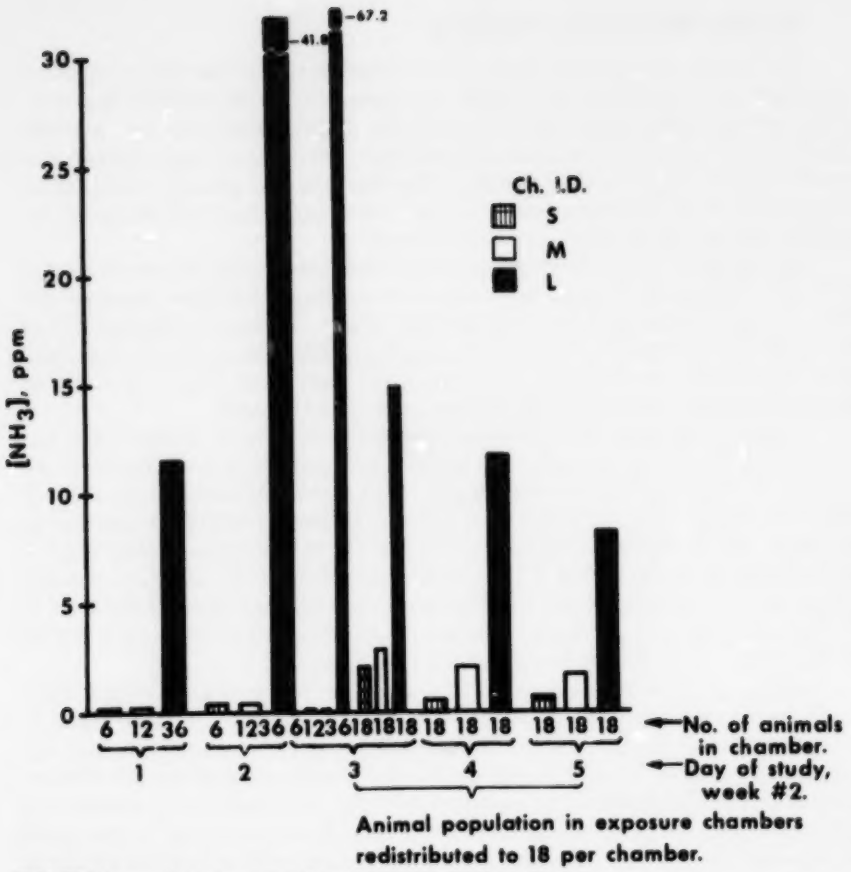

FIGURE2. Ammonia (gas) concentrations in exposure chambers, Phase II.

to prevent the urine and feces of those animals from falling on the animals and cages below and also to deter the decomposition process of the animal refuse producing the ammonia or ammonia-type compounds.

Initially, when the engine was not operating and contributing combustion products to the supply air, the chamber atmospheres showed 1.5 to 2.5 ppm (1.04 to 1.73 mg/m³) ammonia (Fig. 4). (Compare with the same-scale ammonia values in Phase II, Fig. 2). Ammonia gas levels decreased to 0.5 ppm (0.35 mg/m³) and less when the engine exhaust emissions were added to the chambers' air supply and returned to 2 to 3 ppm (1.39 to 2.09 mg/m³) when the engine was turned off on the final (fifth) day. A fresh replacement of the underlay paper on the last day resulted in a definite decrease of ammonia gas concentration in the two chambers.

Figure 5 shows, with one exception, that there was a readily measurable quantity of acid in the particulate, 10-45 mol percent, during the four-day

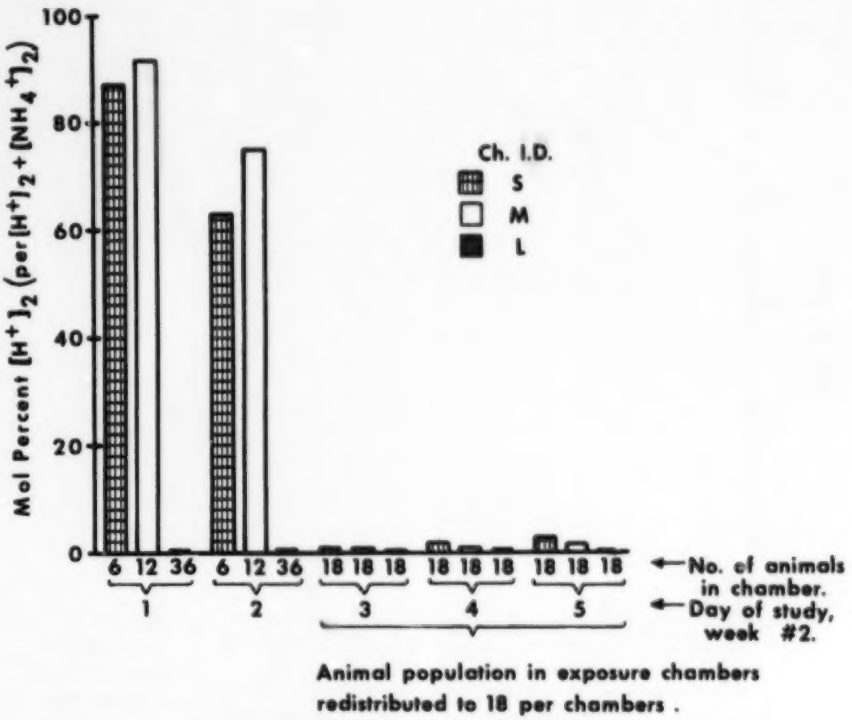

FIGURE 3. Acidity of atmospheric particulate in exposure chambers, Phase II.

period of engine operation. Contrary to expectations, Chamber M, with the somewhat larger complement of animals, usually yielded a more acidic particulate than did Chamber S. The acidity values of Figure 5 are based on calculations from measurement of hydrogen and ammonium ions in the filter extact. The pH measurements, consistently in the 3.5 to 4 range, were much lower than those for the high(er) animal population chambers of Phase II, which were consistently in the 5.5-6.0 range.

Sulfate was the primary and virtually only anionic component of the suspended particulate (aerosol). In the dilution tube, the region of the piping system before the exposure chambers, sulfate concentration levels averaged 5.5 to 6.5 mg/m³ during Phases II and III. Those dilution tube samples were essentially 50 percent sulfuric acid; concentrations of ammonium and nitrate ions were negligible as measured.

Under similar conditions of operations during an earlier study, the acid aerosol generated by the gasoline engine exhaust system with its oxidative

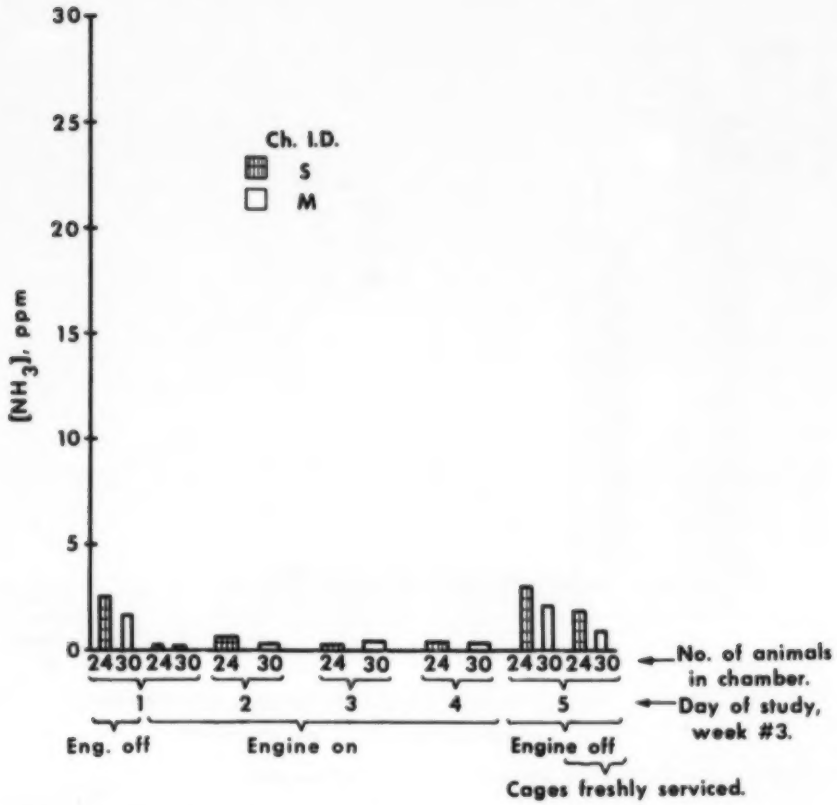

FIGURE4. Ammonia (gas) concentrations in exposure chambers, Phase III.

catalyst converter was predominantly less than 0.25 micrometer mass median diameter in size. The size measurement was established from the very large mass deposition of particulate matter on the back-up filter of a cascade impactor unit.

DISCUSSION OF RESULTS

Sulfuric acid was present in the dilution tube at a concentration averaging 60 μ mol/m^3. From experience with sulfuric acid aerosols and with other particulate in the same size range, it was expected that about one-third of the acid aerosol would be lost in the duct system by sedimentation and diffusion processes as affected by coalescence and impaction conditons, before reaching the exposure chambers. From sulfate values in the dilution tube atmo-

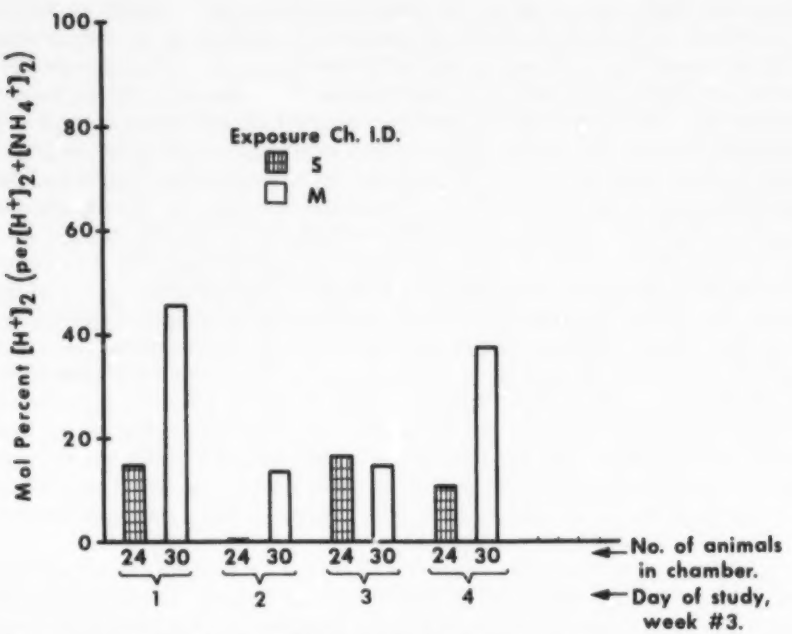

FIGURE 5. Acidity of atmospheric particulate in exposure chambers, Phase III.

sphere and in the exposure chamber atmospheres during Phases II and III, it was determined that a loss of about one-third did occur. Thus, a concentration of about 40 μ mol/m³ sulfuric acid was supplied at the entrance to the exposure chambers.

In Phase I, average ammonia concentrations in the chambers with 6 and 12 rats, S and M, for the week were about 37 and 52 μ mol/m³, respectively. In the chamber with 36 rats, L, the concentration increased from 135 μ mol NH_3/m³ to 1015 μ mol/m³ by the fifth day. On a stoichiometric basis for the reaction of ammonia with sulfuric acid, there would be an excess of about 15-20 μ mol H_2SO_4/m³ in Chambers S and M if the end product were ammonium sulfate, $(NH_4)_2SO_4$. Chamber L, on the other hand, had a considerable excess of ammonia that could readily neutralize all the acid in the atmosphere.

Such neutralization results are evident in Figures 2 and 3 for the Phase II portion of the study. The residual (unreacted) ammonia in Chambers S and M was nearly zero during the first half of the week when six and twelve animals were present in those chambers, as they were during Phase I; correspondingly, the acidity of the particulate product was quite high. The presence of a high level of acid is indicated by the 60-90 mol percent $(H^+)_2$ values seen in Figure 3 for those chambers during the first two days of Phase II as well as in the lower

pH values of approximately 3 measured in the extracts. On the other hand, the extremely high concentrations of ammonia in Chamber L readily neutralized the sulfuric acid in the aerosol and produced a particulate of comparatively neutral ammonium sulfate, as evident in the nearly zero mol percent $(H^+)_2$ values in Figure 3 and the 5.7-5.8 pH values of the extracts. During the latter half of the week, when each chamber contained 18 animals as a result of the redistribution at mid-week, the ammonia production was great enough for nearly all the acid aerosol in the chambers to be neutralized (Fig. 3) and there was an excess of ammonia in the chamber atmospheres (Fig. 2). The ammonia level in Chamber L dropped markedly during the third day after its animal load was reduced from 36 to 18; however, that level had not yet come down by day 5 to those measured in Chambers S and M, even though Chamber L had been given special attention (hosing down with hot water three times daily at 8-hour intervals, and placing aluminum foil shields on top of the animal cages at the lower levels to prevent soiling from the animals in the cages at the upper levels). Chamber L may have been heavily conditioned by adhering particles and trapped solids on surfaces and in corners less acessible to the hosing down cleaning operation, and the animals and cages retained in the chamber may have been heavily saturated and soiled with refuse. The trend of a gradual reduction of ammonia in Chamber L (Fig. 2) approached a concentration level similar to those in Chambers S and M.

In Phase III, an underlay of chemically-treated litter paper was used under the upper-level animal cages in chambers with 24 and 30 rats. The highest ammonia concentration value during the engine-off condition was 3 ppm (2.09 mg/m³ or 122 μ mol/m³) in the chamber with 24 rats, S, early on the fifth day. That value may be compared to the ammonia value stabilized at about 1 ppm (0.70 mg/m³) in the 6- and 12- animal chambers during Phase I (Fig. 1) and 5 ppm (3.48 mg/m³) (not stabilized) in the 36-animal chamber developed by mid-week. On the other hand, where the acid aerosol was present in the atmosphere (during the first four days of the week), the ammonia concentrations for the 24 and 30 animals generally remained under 0.5 ppm (0.35 mg/m³) in Phase III as compared to 0.5 to 3.0 ppm (0.35 to 2.09 mg/m3) for only eighteen animals in the latter half of Phase II. With one exception, there was substantial acidity, up to 45 mole percent, measured in the particulate samples in Phase III (Fig. 5) as compared to no more than 3 mol percent in any of the particulate samples of Phase II for a smaller number of rats, 18, during the latter half of the week.

There was a 25 to 50 percent reduction of ammonia with the installation of fresh litter paper under the animal cages, as seen in the ammonia level changes on the fifth day, when the engine was turned off (Fig. 4).

It was important that the number of experimental animals be limited to a total that was compatible with maintaining the level of the challenging key pollutant(s) in the exposure chamber. In other words, the chamber should not be overloaded with test animals to the extent that the essential nature of the atmosphere is changed by the presence of the animals. Neither should the concentration of the challenging component(s) having potential toxic effects be significantly reduced nor should the concentration of potenially toxic products of the animal-contributing reactants become consequential.

CONCLUSIONS

The findings of the study may be considered in terms of (1) specific application to the process involving acid aerosol generated by an oxidative catalyst in an automobile engine exhaust system and introduced into an animal exposure chamber, and (2) general application to animal exposure studies.

(1.) In the specific case, with a flow of air through the exposure chambers such as 0.31 m^3/min (11 cfm), or one change every four minutes, average servicing for cleanliness (water flushing of the chamber once a day), and the automobile engine operating under such conditions as to generate from its catalyst unit approximately 6 mg/m^3 sulfuric acid in the diluted exhaust supplied by way of the irradiation chambers to the exposure chambers, the data showed that:

a. In order to maintain a predominantly acid particulate, no more than 12 to 18 adult rats should occupy a single 1.25 m^3 (44 cf) chamber unless special provisions are made to reduce the generation of ammonia from the animals.
b. Hosing down the exposure chamber walls and support gratings had little effect upon maintaining the ammonia concentration at a low level.
c. Underlaying the cages with the chemically-treated litter paper had considerable effect in reducing ammonia concentration in the exposure chamber.
d. Perhaps the equivalent of twenty rats could be assigned to a chamber in which the underlay paper is used and cages are changed weekly, to assure maintaining an acidity above 75 mol percent in the particulate of such an exhaust emission supplied atmosphere.

(2.) More generally, it may be concluded that:

a. There is a critical number of animals that can be retained in an exposure atmosphere without significantly changing the concentration of the particular key component under study. A reaction-type change eliminates that component. Of course, consideration has to be given to other components and their products that may be present in sufficient quantity to affect the results.
b. The critical number can vary quite significantly according to (a) animal type, age, size, and condition, (b) cage placement, (c) exposure chamber air flow, temperature, and humidity, (d) atmosphere component type, concentration, and state, and (e) preventive measures to minimize the animal contribution to the atmospheric composition and state.
c. The length of the study can have a serious impact on the component concentrations over an extended period because of the accumulation of trapped waste matter in the chambers, on the cages, and on the animals themselves that does not get removed by simple cleaning operations.

The study demonstrates the value of checking the exposure system before initiating a long-term, involved toxicology study. An investigator should consider performing brief runs under similar test conditions in which animals are used that are of the same species, number, age and size as those intended for the long study. An evaluation of the key atmosphere component(s) may help resolve the design for the full-term study.

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